

Host Proteins Associated with HIV-1

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INTRODUCTION

Retroviruses including HIV-1 assemble their core proteins and genomic RNA in the cytoplasm of the host cell and exit the cell by budding from the plasma membrane. During the budding process, the assembling virus acquires viral surface proteins associated with the host membrane and a lipid envelope (derived from the plasma membrane). The assembly and budding process excludes most host proteins from the surface [1] and the mature virus is often thought of as being composed of only virus-encoded proteins. However, recent evidence strongly suggests that not all host proteins are excluded from the virus and that some host proteins may even be enriched in the mature virus. This brief review will focus on the evidence for host-derived proteins that are now believed to be incorporated into mature HIV-1.

Highly purified preparations of retroviruses usually contain detectable amounts of host cell proteins. These proteins may be associated with the virion itself, or with microvesicles and other membranous particles that co-purify with the virus. Moreover, some host proteins appear to be incorporated into most virions while others may only be incorporated into a small fraction of the bulk virion population. Associations with a small fraction of total virions are not necessarily insignificant, but they imply that the virus has not evolved a specific incorporation process. Conversely, host proteins that are in most or all virions are more likely to be incorporated by a specific viral process. The initial challenge in studying virus/host protein interactions is to distinguish between these alternatives and to definitively identify host proteins specifically incorporated to virus particles.

EXTERIOR PROTEINS

Host membrane proteins are often identified in viral preparations by immunological methods. Whole virus immunoprecipitation or capture methods using antibodies against a host protein strongly suggest a physical association between the host protein and the virus. When these methods are extended to show that most virions are physically associated with a host protein the data support a specific incorporation. This portion of the review will focus on host proteins that have been shown to be physically associated with virions.

Proteins that have been reported to be on the surface of HIV-1 virions are listed in Table 1.

Of the host proteins listed in Table 1, those derived from the major histocompatibility complex (MHC) are the best characterized. Highly purified preparations of HIV-1 produced from lymphoid cells (H9) contain substantial amounts of host histocompatibility proteins, HLA class II-DR (HLA-DR) and HLA class I [2]. Virus immunoprecipitation experiments confirmed that HLA-DR and HLA class I complexes were physically bound on the surface of most or all virions [3]. Quantitation of the amounts of HLA-DR present in the viral preparation suggested that in some cases there may be as many or more HLA-DR molecules on the surface of the virion as there are gp120SU molecules (approximately 216 [10]). Qualitatively, these results have been extended to virus from HIV-1 patient plasma and to SIV grown in HuT78 (L. Arthur NCI-FCRDC, personal communication).

HLA-DR is the predominant subtype HLA class II molecule on the surface of the H9 cell line but lesser amounts of other MHC subtypes such as HLA-DQ and HLA-DP are also present[3]. The presence of the DR-subtype and the relative absence of the DP- and DQ-subtypes in purified virus produced from H9 cells suggests, but does not prove, a selective incorporation of the DR subtype.

Table 1 Exterior Proteins on HIV-1 Virions

Protein	Method		Reference
	Detected	Function in Host	
HLA Class II(α , β)	B,I,C,E,F	MHC Restriction	2,3,4,5,6,7,8,9
HLA Class I	B,I,C,E	MHC Restriction	2,3,4,5,6,8
b2-Microglobulin	B,I,C, E	MHC Restriction	2,3,4,5,8
CD3	E	T Cell Signaling	7
CD4	E	T Cell Signaling	7
CD8	C	T Cell Signaling	4
CD11a/LFA-1	C,E	Cell/Cell Adhesion	4,5,8
CD25	E	Lymphocyte Marker	7
CD30	E	Lymphocyte Marker	7
CD43	C	Lymphocyte Marker	4
CD44	C	Lymphocyte Marker	4,
CD54/ICAM-1	C,E	Cell Adhesion/Signaling	4,5,8
CD63	C,E	Lymphocyte Marker	4,7
CD71	C	Transferrin Receptor	4

B= Biochemically characterized

C=Whole virus capture

E= Immunoelectron microscopy

F= Fluorescence Activated Cell Sorter Analysis

I= Immunoprecipitation

At present, the function of the HLA complexes on the virus is open to speculation. Infectious virus can be produced from lymphoid cells that lack HLA complexes (T2 cells) and cells with reduced levels of HLA-DR (CEM cells) as well as non-lymphoid cells lines that have no HLA class II, such as HeLa and 293. These observations clearly show that the HLA complexes are not strictly required for virus assembly, budding, or infection.

It has been suggested that the HLA class II complexes on the viral surface may offer an advantage to the virus by down-regulating the immune system. Specifically, it is suggested that interactions of virion-associated HLA class II with the T cell receptor (TCR) and gp120SU with CD4 molecules on helper T cells might cause the cells to undergo anergy and apoptosis [3]. The suggestion stems from the fact that normal activation of helper-T cell requires both HLA class II interactions with the TCR (stabilized by CD4 interactions) and additional co-stimulatory factors that are present on an antigen presenting cell. Without a co-stimulatory signal, a T cell with bound TCR undergoes anergy leading to apoptosis [reviewed in 11]. Virions contain HLA class II which can bind TCR [12] but do not contain appreciable amounts of co-stimulatory factors. Thus, it has been suggested that interaction of virions with CD4+ T cells may induce anergy and apoptosis without infection [3,12].

Virus-associated host proteins have important implications for the development of vaccines. Recently it was shown that immunization of macaques with uninfected human T-lymphoid cells which contain cellular antigens elicited a protective immune response against SIV produced from the same cell line [13]. At least part of the protective immune response can be attributed to the human HLA complexes both in the immunogen and on the virus. This was clearly demonstrated by immunizing macaques with purified human HLA complexes and challenging with either SIV from HuT78 cells or SIV from macaque cells. Macaques immunized with human HLA-DR were protected from human-produced SIV but were not protected from macaque-produced SIV [14]. These data show that the protective immune response was elicited by the purified human HLA class II complex and was directed to the human HLA-DR complex on the challenge virus.

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Whole virus capture methods have detected other host proteins on the surface of HIV-1 (Table 1). These include adhesion proteins and cell surface antigens normally involved in T-cell signaling processes. These methods support a physical association of the proteins with the virion but the amounts of antigen per virion or the fraction of total virion particles carrying the antigen is often not clear. However, CD11a (LFA-1), the receptor for ICAM-1, has been detected by a whole virus capture method [4]. A functional role for LFA-1 is suggested by the recent report that a monoclonal antibody to LFA-1 has been shown to enhance plasma neutralization of HIV-1 [15]. The data support the suggestion that LFA-1 is present on most of the virions and that viral associated adhesion proteins may assist in viral attachment and penetration.

INTERIOR PROTEINS

Detection and identification of host proteins by immunoprecipitation or whole virus capture methods fail if the protein is sequestered inside the viral lipid envelope. For this reason, it has been extremely difficult to distinguish between host proteins that are physically located inside the virion and contaminating host proteins that co-purify with the virus. Nevertheless, investigations using molecular biological techniques and protein chemistry methods have shown that host proteins contribute to the internal composition of HIV-1.

Recent studies using molecular biology techniques have shown that cyclophilin A (CyPA), an abundant cytosolic protein, interacts with the p24CA domain in the HIV-1 Gag precursor [16,17,18]. HIV-1 that contained a mutation in Gag (pro222)[17] and HIV/SIV Gag chimeras[18] produced virus-like particles that did not contain CyPA and were non-infectious. These data suggested that CyPA might interact with the Gag precursor during virus assembly and incorporate into the budding virion. Since CyPA is known to catalyze cis-trans isomerization of proline residues, it has been postulated that the protein may function to assist protein folding transitions during early infection events [19].

The presence of CyPA in highly purified HIV-1 has been confirmed by protein chemistry methods [20]. This determination was made possible by using a cloned HIV-1-infected cell line, Clone 4, that produces high levels of replication competent virus without large amounts of microvesicle contamination. Localization of CyPA inside virions was accomplished by employing a nonspecific protease digestion technique on the virus preparation that strips away proteins on the exterior of the lipid bilayer while sparing interior proteins. The digestion alters the sedimentation properties of the virus and microvesicle particles such that they can be separated by sucrose density centrifugation. Viral proteins removed by this process include gp120SU and gp41TM as well as both HLA complexes. Proteins remaining after digestion were subsequently isolated and identified by amino acid sequence analysis. Proteins retained inside the viral lipid envelope include the mature Gag proteins (p17MA, p24CA, p2, p7NC, p1 and p6) and any associated host proteins. Table 2 is a partial list of host proteins that have been identified by this process and are believed to be located inside virus produced on H9 cells or CEM cells. Actin and ubiquitin have been previously detected in purified virion preparations [3].

Table 2 Interior Proteins	
Protein	Host Function
cyclophilin A	protein folding
actin	cytoskeleton
ezrin	cytoskeleton
moesin	cytoskeleton
ubiquitin	protein turnover

Very little is known about the implications of these host proteins in viral processes. Actin is a major component of the cytoskeleton structure and is believed to be important for intracellular transport processes. Ezrin and moesin are closely related proteins and are believed to form associations with

actin and the plasma membrane. It is tempting to speculate that actin, ezrin and moesin may play a role in viral assembly and during the budding process.

Ubiquitin is found in all eukaryotic cells and is known to form multiple covalent complexes with the ϵ -lysine of cellular proteins. Ubiquitin modification is thought to play a role protein turnover by directing proteins to a protease complex but may also have other functions. A covalent complex between ubiquitin and the p6 Gag protein has been purified from HIV-1 and identified by amino acid sequence analysis (Henderson, unpublished data). Only a small fraction (less than 1%) of the total p6 in the virus is complexed with ubiquitin and most of the ubiquitin in the virus is free protein. However, the presence of p6-ubiquitin complex supports the suggestion that ubiquitin is inside the virion. Ubiquitin has been observed in highly purified preparations of many other types of retrovirus (Henderson, unpublished observations). These observations suggest that ubiquitin may play a fundamental role in retroviral replication but at present the role, if any, is unknown.

CONCLUSION

The study of host proteins associated with virions should lead to a better understanding of the interactions that are required for steps in the virus life-cycle. The presence of host proteins in virions may reflect a requirement for their function in early infection events, as CyPA may, or are a remnant of an interaction between viral and host proteins. A better appreciation for the role of these proteins should lead to better intervention for AIDS.

References

- [1] Zavada, J. (1982) The pseudotypic paradox. *J. Gen. Virol.* **63**:15–24.
- [2] Henderson, L.E., Sowder, R., Copeland, T.D., Oroszlan, S., Arthur, L.O., Robey, W.G., and Fischinger, P.J. Direct identification of class II histocompatibility DR proteins in preparations of human T-cell lymphotropic virus type III. *J. Virol.* **61**:629–632.
- [3] Arthur, L.O., Bess, J.W., Jr., Sowder, R.C. II, Benveniste, R.E., Mann, D.L., Chermann, J.-C., and Henderson, L.E. (1992) Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. *Science* **258**:1935–1938.
- [4] Orentas, R.J. and Hildreth, J.E.K. (1993) Association of host cell surface adhesion receptors and other membrane proteins with HIV and SIV. *AIDS Res. Hum. Retroviruses* **9**:1157–1165.
- [5] Capobianchi, M.R., Fais, S., Castilletti, C., Gentile, M., Ameglio, F., and Dianzani, F. (1994) A simple and reliable method to detect cell membrane proteins on infectious human immunodeficiency virus type 1 particles. *J. Inf. Dis.* **169**:886–889.
- [6] Gelderblom, H., Reupke, H., Winkel, T., Kunze, R., and Pauli, G. (1987) MHC-antigens: constituents of the envelopes of human and simian immunodeficiency viruses. *Z. Naturforsch* **42**:1328–1334.
- [7] Meerloo, T., Parmentier, H.K., Osterhaus, A.D.M.E., Goudsmit, J., and Schuurman, H.-J. (1992) Modulation of cell surface molecules during HIV-1 infection of H9 cells. An immunoelectron microscopic study. *AIDS* **6**:1105–1116.
- [8] Meerloo, T., Sheikh, M.A., Bloem, A.C., de Ronde, A., Schutten, M., van Els, C.A.C., Roholl, P.J.M., Joling, P., Goudsmit, J., and Schuurman, H.-J. (1993) Host cell membrane proteins on human immunodeficiency virus type 1 after in vitro infection of H9 cells and blood mononuclear cells. An immuno-electron microscopic study. *J. Gen. Virol.* **74**:129–135.
- [9] Schols, D., Pauwels, R., Desmyter, J., and De Clercq, E. (1992) Presence of class II histocompatibility DR proteins on the envelope of human immunodeficiency virus demonstrated by FACS analysis. *Virology* **189**:374–376.

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- [10] Gelderblom, H.R., Ozel, M., Winkel, T., Morath, B., Grund, C., and Pauli, G. (1991) Ultrastructural studies on lentiviruses in: *Accessory cells in HIV and other Retroviral infections: morphological and functional aspects*. P. Racz, C.D. Dijkstra, J.-C. Gluckman, Eds. Karger, Basel, Switzerland. pp. 50–68
- [11] Ameisen J.C. (1995) HIV infection and T-cell death. in *Apoptosis and the immune response*. Ed. C.D. Gregory, Wiley-Liss, New York, New York, pp.115–142.
- [12] Rossio, J.L., Bess, J., Jr., Henderson, L.E., Cresswell, P., and Arthur, L.O. (1995) HLA class II on HIV particles is functional in superantigen presentation to human T cells: implications for HIV pathogenesis. *AIDS Res. Hum. Retroviruses*. In press.
- [13] Stott, E.M. (1991) Anti-cell antibody in macaques. *Nature(London)* **353**:393.
- [14] Arthur, L.O., Bess, J.W., Jr., Urban, R.G., Strominger, J.L., Morton, W.R., Mann, D.L., Henderson, L.E., and Benveniste, R.E. (1995) Macaques immunized with HLA-DR are protected from challenge with simian immunodeficiency virus. *J. Virol.* **69**:3117–3124.
- [15] Gomez, M.B. and Hildreth, J.E.K. (1995) Antibody to adhesion molecule LFA-1 enhances plasma neutralization of human immunodeficiency virus type 1. *J. Virol.* **69**:4628–4632.
- [16] Luban, J., Bossolt, K.L., Franke, E.K., Kalpana, G.V., and Goff S.P. (1993) Human immunodeficiency virus type 1 gag protein binds to cyclophilins A and B. *Cell* **73**:1067–1078.
- [17] Franke, E.K., Yuan, H.E.H., and Luban, J. (1994) Specific incorporation of cyclophilin A into HIV-1 virions. *Nature(London)* **372**:359–362.
- [18] Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C.T., Sodroski, J., and Gottlinger, H.G. (1994): Functional association of cyclophilin A with HIV-1 virions. *Nature(London)* **372**:363–365.
- [19] Klasse, P.J., Schulz, T.F., and Willison, K.R. (1993) Cyclophilins unfold the gag? *Nature(London)* **365**:395–396.
- [20] Ott, D. E., Coren, L.V., Johnson, D.G., Sowder, R.C., II, Arthur, L.O., and Henderson, L.E. (1995) Analysis and localization of cyclophilin A found in the virions of human immunodeficiency virus type 1 MN strain. *AIDS Res. Hum. Retroviruses*. **11**:1003–1006.